3-2017

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF RELEVANT *Sclerotinia sclerotiorum* ISOLATES

Thomas J.J. Miorini
University of Nebraska-Lincoln, tmiorini3@unl.edu

Anthony G. Pannullo
University of Nebraska-Lincoln, apannullo2@unl.edu

T. Hornby
University of Nebraska-Lincoln

R. B. Higgins
University of Nebraska-Lincoln

Sydney E. Everhart
University of Nebraska-Lincoln, everhart@unl.edu

See next page for additional authors

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF RELEVANT *Sclerotinia sclerotiorum* ISOLATES

T.J.J. Miorini, A. Pannullo, T. Hornby, R.B. Higgins, S.E. Everhart, and J.R. Steadman

University of Nebraska, Department of Plant Pathology, Lincoln, NE 68583-0722

Knowledge of pathogen population structure is useful to identify isolates for screening cultivars and lines for resistance. For *S. sclerotiorum*, causal agent of white mold in more than 400 plant species, including common bean and soybean, breeding for resistance is particularly challenging. The objective of this study was to characterize phenotypic and genotypic variation of *S. sclerotiorum* isolates from soybean production areas of the U.S.A. (15), Brazil (75), and Argentina (5) to compare them with 366 isolates from dry bean characterized previously (Everhart *et al*., 2016).

Genotyping **–** DNA of 95 isolates was extracted and genotyped at 11 SSR loci (Sirjusingh, and Kohn, 2001). Identified were 92 multilocus genotypes, with only four represented by more than one isolate. Our results showed these isolates had greater genotypic richness and diversity compared with the 366 isolates genotyped previously (Everhart *et al.,* 2016). Pairwise genetic distances between isolates was estimated using Bruvo's distance, which utilizes a stepwise model of mutation. The matrix of pairwise distances was used to construct a minimum spanning network (MSN); using the R package *poppr* (Fig. 1). Within the MSN was a core set of 12 MLG that were closely related (thick lines in MSN) and from six states in Brazil. These results are consistent with that expected for a soilborne organism, such as *S. sclerotiorum*.

Table 1. Number of isolates (N), number of multilocus genotypes (MLG) and genotypic diversity (*h*) within populations of *S. sclerotiorum*

Populations	N	MLG	*h*
Nebraska	14	14	0.929
Argentina	5	5	0.800
Bahia	14	13	0.918
Rio Grande do Sul	16	16	0.938
Paraná	16	16	0.938
Mato Grosso do Sul	6	6	0.833
Goiás	17	15	0.927
Minas Gerais	7	7	0.857

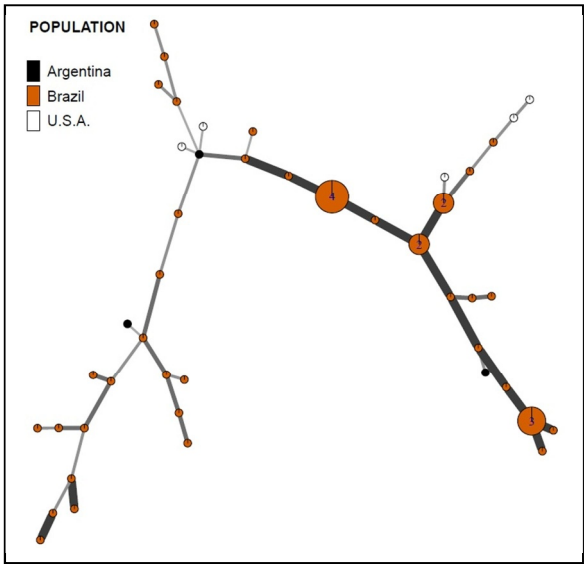

Figure 1. MSN generated for 52 isolates using distance-based analysis methods.

Mycelial compatibility group – A sub-set of 69 isolates were paired and grown on DS medium and evaluated for compatibility after 10-days growth. Identified were 25 MCGs, with 44% of these groups represented by only one isolate (Fig. 2). The most abundant MCG was represented by 16 isolates from Brazil that were geographically partitioned into 10 regions from 5 states. One MCG was identified in the U.S.A. (one isolate; Bellwood, NE) and Brazil (two isolates; Chapadão do Sul, Mato Grosso do Sul). Within the same field in Mead, NE and Auburn, NE were two different MCGs, each represented in both fields.

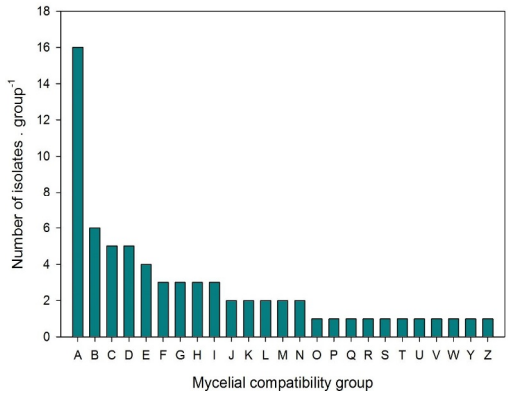

Figure 2. Frequency distribution of the 25 MCG identified (A-W, Y, Z) among the 69 isolates characterized in this study.

Phenotyping – Aggressiveness of 70 isolates was determined using a detached leaf bioassay (DLB), wherein leaves of partially resistant soybean cultivar "Dassel" were inoculated with an agar plug of mycelium. The three youngest and fully expanded leaves were collected at 21, 28, and 35 days after emergence and lesion areas observed 48 hours after inoculation. Preliminary analysis (Fig. 3) showed leaf age influenced results of aggressiveness assays, with larger necrotic lesion areas observed on leaves of younger plants (21 days). Necrotic area varied among 70 isolates.

Importance for breeding programs – our current work will enable selection of isolates that are representative of the *S. sclerotiorum* populations throughout deployment regions. Differences in isolate aggressiveness and genetic variation have identified *S. sclerotiorum* that can be used for cultivar evaluation studies.

Conclusions – Preliminary results showed more variation in isolates from South American soybean than those genotyped previously in dry bean (Everhart *et al*., 2016); necrotic lesions on leaves decreased with increasing plant age. The overall approach we used in our soybean/dry bean research is applicable to facilitate identification of white mold resistance in other susceptible crops including canola, pulses, and sunflower.

Future Direction – MCGs and MLGs of isolates in the present study will be used for comparison with 366 isolates genotyped previously; validation of fungicide sensitivity methods is currently underway by assessing closeness of estimate to true 50% inhibitory dose. Further analysis of isolate aggressiveness is also underway.

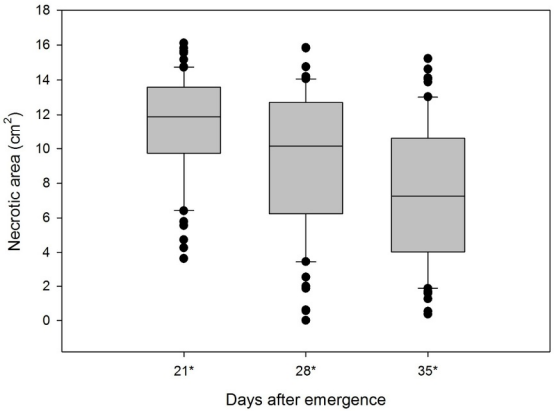

Figure 3. Necrotic lesion area (cm^2) formed on soybean leaves collected at 21, 28, and 35 days after emergence and lesion areas observed 48 hours after inoculation. Each plot represents 10 repetitions of 70 isolates (700 total).

REFERENCES

Everhart, S.E., B.S. Amaradasa, R. Jhala, R. Higgins, and J.R. Steadman. 2016. Population structure and fungicide sensitivity of 366 *Sclerotinia sclerotiorum* isolates from dry common bean. *Bean Improv. Coop.* 59:131-132.

Sirjusingh, C. and L.M. Kohn. 2001. Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum. Molecular Ecology* 1:267-269.